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Zelder, Felix Hubertus

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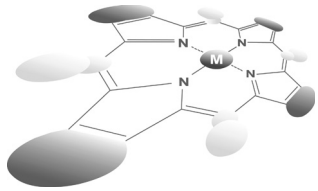

Modified vitamin B_{12} derivatives with a peptide backbone for biomimetic studies and medicinal applications

Felix Zelder*◊

Department of Chemistry, University of Zurich, Switzerland

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ABSTRACT: This short review highlights the author's group research on modified vitamin B_{12} derivatives with a peptide backbone as (1) inhibitors of B_{12}-dependent enzymes and as (2) models of cofactor B_{12}-protein complexes.

KEYWORDS: vitamin B_{12}, peptide, model, mimic, inhibitor, drug, antivitamin, cofactor, enzyme.

INTRODUCTION

Cobalamin (Cbl) represents the structurally most complex member of the porphyrinoid family. It consists of a central cobalt ion, a tetradentate corrin macrocycle, a dimethylbenzimidazole (Dmbz) base that is connected to the *f*-side chain of the corrin by a α-ribazole containing backbone and an upper, axially coordinating ligand (Fig. 1) [1, 2].

MethylCbl (MeCbl) and adenosylCbl (AdoCbl) (Fig. 1) are mainly known as organometallic cofactors in methyl transfer reactions [1, 2] and radical rearrangements [3]. Only recently it has been demonstrated that AdoCbl employs even more versatile functions in biological systems and acts as a light sensing system in bacteria such as *Myxococcus xanthus* [4, 5].

This exciting discovery suggests that other important, yet unknown roles of Cbls in biological systems will be unraveled in the future [6].

Reactivity, properties and functions of Cbls in biological systems are related to their intrinsic redox and coordination properties at the cobalt center and are skillfully fine-tuned by the apoenzymes [3]. Cbls are encountered as octahedral complexes in the Co^{III} state, as square pyramidal Co^{II}-, or square planar Co^{I}-forms after one- or two-electron reductions. In the complexes, the intramolecular bound Dmbz base is either coordinated ("base-on") to the metal center or in an unbound configuration ("base-off"). The base-on/base-off equilibrium of Cbls (Scheme 1 *middle*) plays important roles for (i) B_{12} uptake [7], (ii) B_{12} metabolism [8] and (iii) cofactor's reactivity in enzymatic reactions [9].

For example, a switch from base-on co(II)balamin to its base-off form (Scheme 1) shifts the pH-independent standard potential of the Co^{II}/Co^{I} reduction from -0.85 V *vs*. SCE to -0.74 V *vs*. SCE by ~110 mV more positive and hence, reduction is thermodynamically favored [10]. This behavior can be explained by the weaker σ-donating properties of the aqua ligand compared to the intramolecular coordinated Dmbz base in the square pyramidal complexes. The thermodynamically more accessible base-off co(II)balamin is therefore probably encountered during reductive adenosylation with ATP to AdoCbl catalyzed by adenosyltransferase in the mitochondria [8].

In certain cofactor–protein complexes such as MeCbl-dependent methione synthase (MetH) as well as AdoCbl-dependent methylmalonyl CoA mutase (MCM), the replacement of the intramolecular bound Dmbz nucleotide loop of Cbls by a protein's histidine (His) residue is observed [11, 12]. The cobalt-coordinated His is further embedded in a hydrogen bonding network, together with aspartic acid (Asp) and either lysine (for MCM) or serine (for MetH) [13].

This particular mode of cofactor anchoring has similarities to His-on coordinated heme proteins in

◊ SPP full member in good standing

*Correspondence to: Dr. F. Zelder, Department of Chemistry, University of Zurich, Winterthurerstr. 190, CH-8057 Zurich, tel.: +41 44 635 4624, fax: +41 44 635 6802, email: felix.zelder@chem.uzh.ch www.felix-zelder.net.

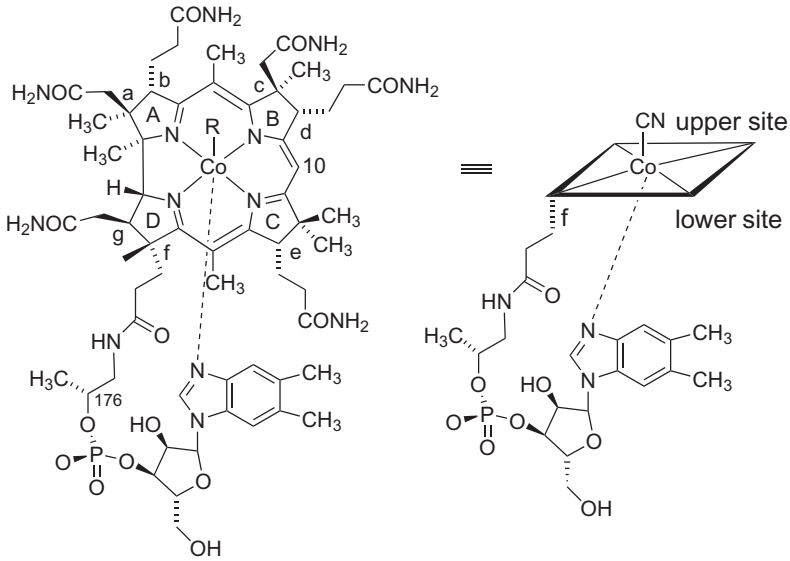
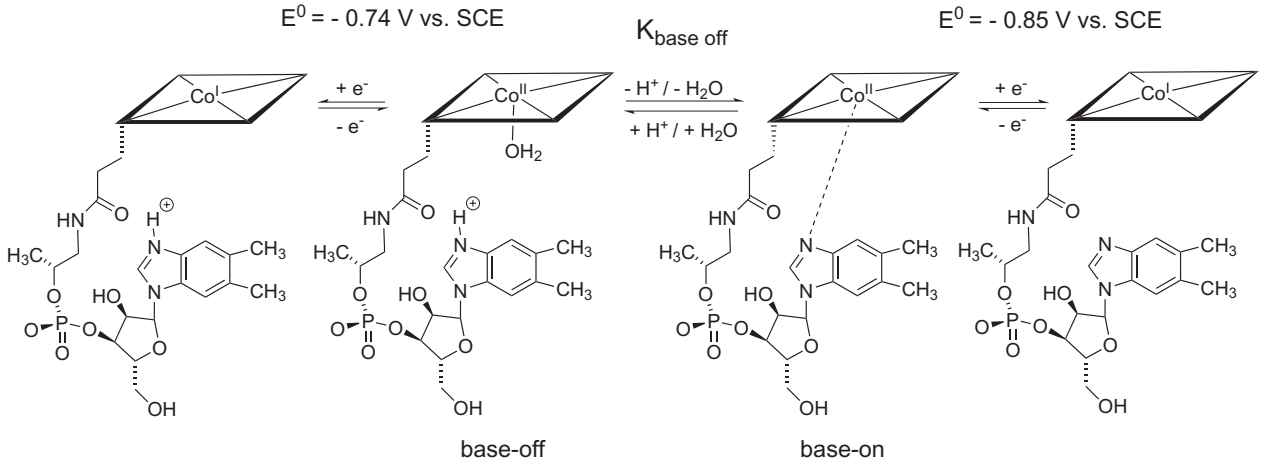


Fig. 1. *Left*: Structures of Cbls (*R* = CN: cyanoCbl (B_{12}; **1**); *R* = CH_3: MeCbl; *R* = adenosyl: AdoCbl). *Right*: Schematic representation of **1**



Scheme 1. Base-on/base-off equilibrium of co(II)balamin (*middle*) and the corresponding pH-independent one-electron reductions to the Co^{I}-forms

globins, cytochrome *c* or horseradish peroxidase [14]. In these cofactor–protein complexes, partial deprotonation of the lower coordinated His-ligand enhances the electron density at the iron center and the opposite located axial ligand ("push effect") [15, 16]. In contrast to heme proteins, the roles of the lower coordinated protein histidine ligand and the hydrogen bonding network for catalysis in base-off/His-on Cbl-protein complexes have been controversially discussed [17]. Mutation studies with MetH revealed a significant decrease of enzyme activity when either His or Asp were replaced by other amino acids in the hydrogen bond network [18]. A more recent study suggested the stabilization of a catalytically active Co(II) intermediate in "His-on" AdoCbl-dependent isomerases by a less basic His ligand [17]. Clearly, further studies on Cbl-protein complexes and thoroughly designed model complexes are required to better understand the roles and impacts of the first and second coordination sphere of Cbls in enzymatic reactions.

This short review focuses on the author's group efforts in the development of modified B_{12} derivatives with a peptide backbone as models of cofactor–protein complexes as well as for medicinal and biological applications. For more comprehensive insight into the topic including the uptake, metabolism and reactivity of Cbls in biological systems [3, 7, 8, 17, 19–21], the development of B_{12} derivatives as biomimetic models [22–28], or the use of Cbls for medical [2, 29–40] and analytical applications [41–46], the interested reader is

referred to the original literature as well as to several excellent reviews and book chapters [2, 3, 8, 47–51].

Modified vitamin B_{12} derivatives with a peptide backbone

In 2010, my group reported on a new class of modified B_{12} derivatives with a peptide backbone containing a Dmbz base (Fig. 2b) [52, 53]. This structural motif was introduced into the B_{12} scaffold in order to control intramolecular coordination of the Dmbz to the cobalt center of the corrinoid. In particular, we speculated that pertubation of the base-on/base-off trigger may lead to "dysfunctional" B_{12} analogs with potential antivitamin B_{12} activity [29]. With this general idea in mind, we decided to replace the ribose phosphodiester moiety of Cbls with peptide mimics having the same number of atoms between the corrin macrocycle and the nitrogen donor of the Dmbz ligand (Fig. 2b). The peptide mimic of the semi-artificial Cbl is attached to the *f*-side chain of the precursor cobyric acid (Fig. 2a) and consists of three different subunits: (i) an ethylenediamine, (ii) an amino acid and (iii) an acyl modified Dmbz base. The modular composition of the peptide linker allows rapid and straightforward structural modifications and hence seems to be optimally suited for modulating the strength of intramolecular coordination in the semi-artificial system.

The structural motif for backbone modification of Cbls was inspired by the development and applications of peptide nucleic acids (PNAs) which represent powerful analogs of deoxyribonucleic acids (DNA) [54]. In the prototype peptide B_{12} derivative $\mathbf{2}^+$, the peptide loop of the compound contains an ethylenediamine and a glycine (Fig. 2).

Similarly to B_{12}, compound $\mathbf{2}^+$ is present in its base-on form at pH 7 and 23 °C, however spectrophotometric pH titrations revealed that the base-on form is destabilized by a factor of ~20 compared to B_{12}. This property of $\mathbf{2}^+$ is indicated by a $pK_{base\text{-}off}$ value of 1.4 instead of 0.1 for B_{12} [12, 52]. Introducing proline as a more rigid *u*-turn mimic instead of glycine into the artificial linker of peptide B_{12} $\mathbf{4}^+$ (Fig. 2b) led to a three times greater stabilization of the base-on form ($pK_{base\text{-}off\ (\mathbf{4}^+)} = 1.0$) compared to $\mathbf{2}^+$. A remote methyl group located at position C176 of the backbone caused the most interesting effect in this study. Whereas $\mathbf{5}^+$ (Fig. 2) with an *R*-configured methyl group stabilized the base-on form by a factor of two ($pK_{base\text{-}off\ (\mathbf{5}^+)} = 0.6$), its epimer $\mathbf{6}^+$ (Fig. 2b) showed the opposite behavior and led to a significant 10-fold destabilization ($pK_{base\text{-}off\ (\mathbf{6}^+)} = 1.6$). This strong effect seems surprising considering that the equipment of the backbone with a remote methyl group is ten atoms away from the Co ion. Conformational analysis of the artificial loop revealed that the methyl group effect at C176 is probably best rationalized by an additional gauche effect in the base-on form of $\mathbf{6}^+$ compared to $\mathbf{5}^+$, as schematically depicted in Scheme 2. This type of analysis of the backbone of Cbls has been proposed earlier by Eschenmoser and later successfully applied by Kräutler and co-workers [55, 56]. Although final proof for the effect of a remote methyl group in peptide B_{12}s by crystal structure analysis of $\mathbf{5}^+$ and/or $\mathbf{6}^+$ is unfortunately still lacking, the data suggest that artificial peptide loops indeed represent versatile mimics of the α-ribonucleotide backbone of B_{12}.

Modifications of the backbone structure in peptide B_{12}s had also an influence on the redox properties of the Co^{III} center. In general, it was observed that the more stable the base-on form of a peptide B_{12} derivative, the more difficult it was to reduce from Co^{III} to Co^{II}. Although the effect was rather small ($\Delta V = 57$ mV), a linear relationship between the cathodic reduction potential of the $Co^{III/II}$ reduction and the $pK_{base\text{-}off}$ values was observed [52].

In contrast to modifications at the *f*-side chain of Cbls, we also expanded the concept to peptide mimics of the upper adenosyl ligand. PeptidoadenosylCbl **7** (Fig. 3) was employed in enzymatic studies with glutamase mutate from *Clostridium cochlearium* as an AdoCbl mimic lacking the 2′-OH and 3′-OH functional groups of the adenosyl ligand. Although **7** still binds strongly to the holoenzyme, it did not show any catalytic activity.

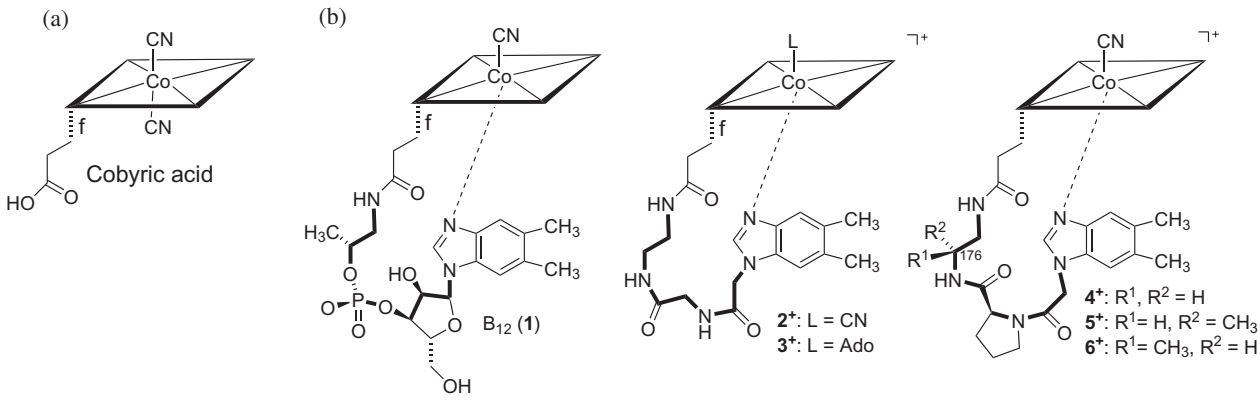
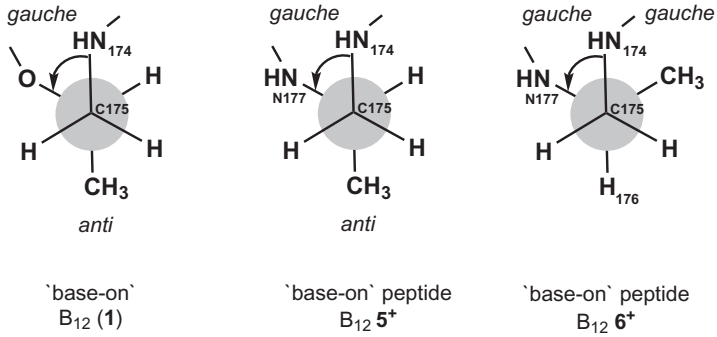


Fig. 2. (a) Cobyric acid. (b) The backbone of B_{12} (**1**; left) compared to that of peptide B_{12}s ($\mathbf{2}^+$, $\mathbf{3}^+$; middle). Peptide B_{12}s $\mathbf{4}^+$–$\mathbf{6}^+$ are shown on the right



Scheme 2. Qualitative conformational analysis of the effect of the methyl group at C176 by using idealized conformations around the C175–C176 bond. Left: 'Base-on' B_{12} (**1**) with one destabilizing *gauche* interaction. Middle: 'Base-on' peptide B_{12} $\mathbf{5}^+$ with one destabilizing gauche interaction. The methyl group at C176 is *anti*. Right: 'Base-on' peptide B_{12} $\mathbf{6}^+$ with two destabilizing gauche interactions. The H at C176 is *anti* [52]

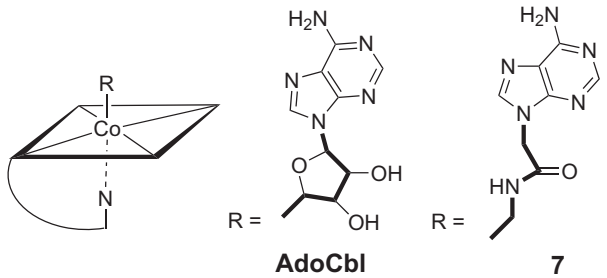


Fig. 3. Comparison of the structures of AdoCbl and peptidoadenosylCbl **7**

In combination with other control experiments, this result underscored the important roles of the 2′-OH and 3′-OH of the ribose moiety of AdoCbl during generation of the Ado radical and hydrogen abstraction from the substrate in enzymatic catalysis [57].

Peptide B_{12} derivatives as antivitamin B_{12} derivatives

In the last decade, the development of structurally modified B_{12} derivatives with antivitamin activity has regained considerable attention [20, 30, 33, 58, 59]. Antivitamins are molecules that diminish or abolish specific functions of vitamins [29, 59, 60] potentially having future impact as new anticancer and antibiotic agents [2].

The impact of structural modification of the backbone in peptide B_{12}s on the biological activity of the natural product was tested in enzymatic as well as bacterial growth studies in my group. The artificial cofactor $\mathbf{3}^+$ (Fig. 2) was applied in an enzymatic assay with glutamate mutase from *Clostridium cochlearium* [20]. Similar apparent values for the Michaelis–Menten constants of $\mathbf{3}^+$ and AdoCbl suggest comparable affinities of the cofactors to the enzyme and demonstrate that both cofactors were able to reconstitute hologlutamate mutase. The artificial cofactor $\mathbf{3}^+$ showed a ten times lower catalytic efficacy ($k_{cat}/K_m = 0.26 \times 10^{-6}$ s^{-1} M^{-1}) compared to the natural cofactor AdoCbl ($k_{cat}/K_m = 2.38 \times 10^{-6}$ s^{-1} M^{-1}). A strongly reduced activity was also observed in bacterial growth studies with *L. leichmannii* applying the related cyano-derivative $\mathbf{2}^+$ (Figs 2 and 4).

A residual growth promoting behavior indicates that despite its severe structural modification, $\mathbf{2}^+$ is still recognized as Cbl, taken up, metabolized and enzymatically active in the microorganism. Nevertheless, competition studies with B_{12} and $\mathbf{2}^+$ demonstrated that peptide B_{12} $\mathbf{2}^+$ antagonizes the natural vitamin with an IC_{50} value of 2 μM after 10 h (Fig. 4).

Considering that only the prototype peptide B_{12} $\mathbf{2}^+$ was tested so far, the results seem to be very promising for developing even more efficient B_{12} antivitamins in the future.

Peptide B_{12} derivatives in biomimetic studies

His-on cofactor anchoring is observed in base-off/His-on cofactor B_{12}-protein complexes such as MetH [9]. Considering similarities to heme proteins, remote control of the lower-coordinated His ligand on reactivity at the opposite site seems therefore attractive for fine-tuning catalysis in cofactor B_{12}-catalyzed reactions [9]. In particular, partial deprotonation of the His ligand through a protein hydrogen bonding network has been proposed to reversibly stabilize and destabilize the Co^{III}-state during catalysis [13, 18], but this mechanism is still controversially discussed [17, 61].

My group develops and studies homogenous and immobilized peptide B_{12} derivatives as structural models of His-on configured Cbl-protein complexes and

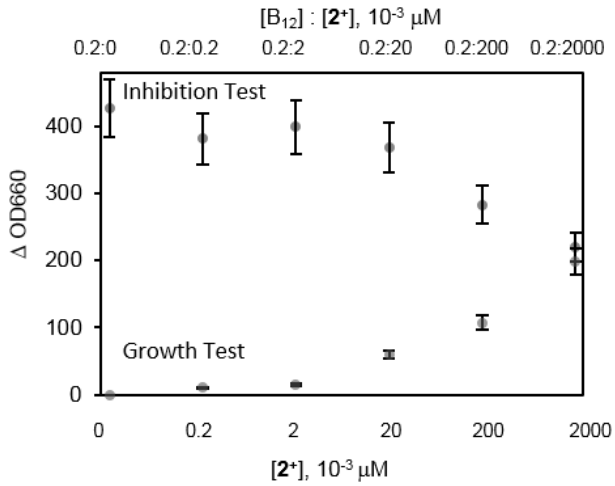
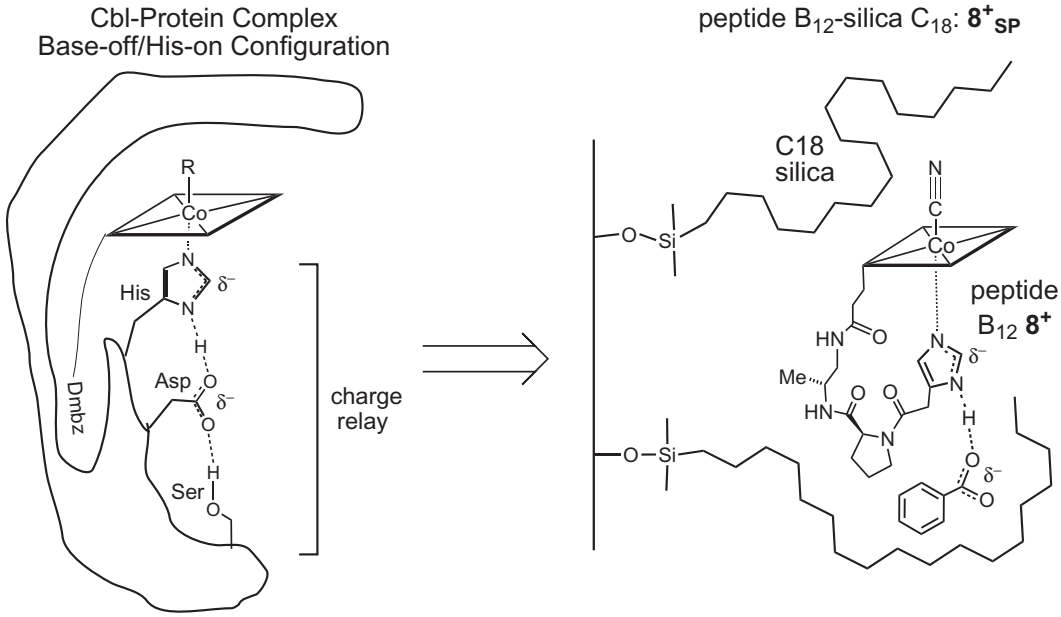


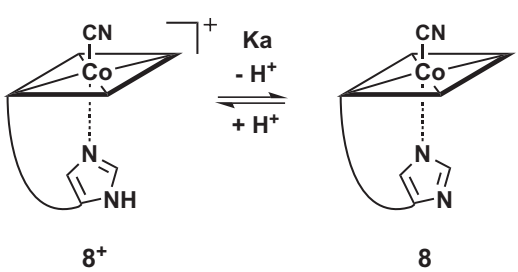
Fig. 4. Inhibition of growth of *L. leichmannii* in the presence of B_{12} and peptide B_{12} $\mathbf{2}^+$ (Inhibition Test) compared to the growth supporting character of $\mathbf{2}^+$ alone (Growth Test) [20]



Scheme 3. *Left:* Cbl bound to the regulatory triad His–Asp–Ser in base-off/His-on Cbl-protein complexes. *Right:* The corresponding biomimetic supramolecular model $\mathbf{8}^{+}_{SP}$ with imidazole hydrogen-bonding interactions [62]

analyzes the influence of lower ligand modulation on the properties of the biomimetic model. The latter consists of a peptide B_{12} derivative that is immobilized on a C18 solid phase (Scheme 3). Peptide B_{12} $\mathbf{8}^{+}$ simulates the His-coordinated cofactor and consists of a corrin macrocycle, a peptide loop terminated with a His instead of a Dmbz base as lower coordinating ligand, and a cyano group on the upper, opposite axial site.

Structural modelling with QM/MM calculations supported by characterization with 2D-ROESY NMR suggested the imidazole backbone derivative $\mathbf{8}^{+}$ as a close biomimetic model of His-on-configured Cbl-protein complexes [62]. Spectrophotometric pH titrations of $\mathbf{8}^{+}$ indicated a pK_a for the coordinated imidazole ligand of 10.8 (Scheme 4), that is approximately four pH units lower than in free imidazole (pK_a = 14.5) [63]. Cyclovoltammetric studies with $\mathbf{8}^{+}$ and **8** strikingly demonstrated the influence of the deprotonation of the nitrogen atom of the coordinated imidazole ligand on the redox properties at the Co^{III} ion. A substantial cathodic shift of ~ -200 mV for the Co^{III}/Co^{I} couple was



Scheme 4. pH equilibrium between $\mathbf{8}^{+}$ and **8**

observed upon deprotonation of $\mathbf{8}^{+}$ to **8**. Deprotonation of the coordinated His ligand of $\mathbf{8}^{+}$ leads to a stronger σ-donating ligand that shifts more electron density to the Co^{III} ion and hence makes its reduction thermodynamically unfavored. Deprotonation of the imidazole ligand to a more basic imidazolate is also reflected in a bathochromic shift of the α-band in the UV-vis spectrum of **8** compared to $\mathbf{8}^{+}$ as observed for Cbls with other axially coordinating ligands [40, 64].

Immobilization of $\mathbf{8}^{+}$ on hydrophobic C18 silica to $\mathbf{8}^{+}_{SP}$ mimics the hydrophobic binding pocket of proteins (Scheme 3). The reflectance spectrum of $\mathbf{8}^{+}_{SP}$ resembled that of base-on $\mathbf{8}^{+}$ under homogenous conditions. The addition of toluene, a solvent that lacks any H-bond acceptor capability to the system, did not lead to any spectral changes. However, addition of benzoate (tetrabutylammonium salt) to this solvent then led to a slight but characteristic shift in the reflectance spectrum. This behavior resembles the spectral changes upon (partial) deprotonation of $\mathbf{8}^{+}$ under homogenous conditions and suggests hydrogen bonding between benzoate and the intramolecularly bound imidazole ligand of $\mathbf{8}^{+}_{SP}$ as schematically depicted in Scheme 3 (*right*). These model studies under homogenous and heterogeneous conditions strongly suggest that partial deprotonation of a coordinated His ligand in Cbl-protein complexes indeed represents a powerful tool to fine-tune the redox chemistry at the Co ion during enzymatic catalysis. Having this unprecedented supramolecular model in hand, we plan to expand the program and study the catalytic activity of immobilized organometallic AdoCbl and MeCbl in organic transformations.

CONCLUSIONS

The development of modified B_{12} derivatives attracts attention for fundamental research, but also for applications in medicine, biology, catalysis and analytical science. Backbone-modified peptide B_{12} derivatives with tunable coordination and redox properties represent a versatile class of Cbls for applications in biology and biomimetic chemistry. Pioneering studies with peptide B_{12} derivatives demonstrated promising inhibitory potential and suggest medicinal potential as antibacterial and antiproliferative agents after further optimization.

Immobilized supramolecular peptide B_{12} assemblies represent powerful structural models of His-on Cbl-protein complexes and help to unravel mechanisms in B_{12}-catalyzed enzymatic reactions. Evidently, backbone modified corrinoids exhibit great potential in different fields that have not yet been exploited sufficiently.

Acknowledgments

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